

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gladwin *et al.*

Application No. 10/563,683

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Confirmation No. 3225

SUBMITTED VIA EFS

For: USE OF NITRITE SALTS FOR THE
TREATMENT OF CARDIOVASCULAR
CONDITIONS

Examiner: Anna Pagonakis

Art Unit: 1614

Attorney Reference No. 4239-67618-07

COMMISSIONER FOR PATENTS
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DECLARATION OF DR. LOUIS IGNARRO UNDER 37 C.F.R. § 1.132

I, Louis Ignarro, Ph.D., declare as follows:

1. I have no financial interest in the above referenced patent application and I am not a listed inventor of the invention disclosed in the above referenced patent application.

2. A copy of my *curriculum vitae* is attached hereto as **Exhibit A**. At present, I hold a position as professor of pharmacology at the UCLA School of Medicine's department of molecular and medical pharmacology in Los Angeles, California. I have had ____ years of experience in research including work on the physiological effects of nitric oxide and inorganic nitrite and particularly the effects of these molecules on vascular tone. In 1998, I received the Nobel Prize in medicine or Physiology for research I conducted on this topic. I have published over ____ scientific articles in scientific journals and books. By virtue of my education, training, and professional experience, I am knowledgeable about nitric oxide donors, the physiology and biology of vasodilation, and the effects of various compounds on vasodilation.

3. I am an editor for the Proceedings of the National Academy of Sciences of the United States of America (PNAS), which is a scientific journal published by the National Academy of Sciences of the United States of America. The scientific community regards PNAS as a highly

respected and prominent journal that publishes original scientific works. As such, scientific researchers are likely to read and trust articles published in PNAS that relate to their field of study.

4. I have read Modin *et al.*, *Acta Physiol Scand.*, 171:9-16, 2001 (attached hereto as **Exhibit B**) and familiarized myself with the teachings therein.

5. The experimental model used by Modin *et al.* is a poor model for predicting *in vivo* responses to sodium nitrite, because this *ex vivo* model utilizes excised rat aorta that is maintained in a modified krebs solution of neutral or acidified pH. Most, if not all, of the regulatory factors present in blood that play a physiological role in the vasodilation process are absent from the experimental paradigm used by Modin *et al.*.

6. I believe that Modin *et al.* does not teach that non-acidified inorganic nitrite is a vasodilator at concentrations of 25 μM or less, *in vitro* or *in vivo*. Reading Figure 2 of Modin *et al.*, I would not have been led to believe that inorganic nitrite, when applied in a neutral buffer, is an effective vasodilator of isolated segments of rat aorta at concentrations of 25 μM or less. I do not believe that a scientist working in the nitric oxide field would believe that the Modin *et al.* reference teaches that non-acidified inorganic nitrite is a vasodilator at concentrations of 25 μM or less, *in vitro* or *in vivo*.

7. I edited for publication in PNAS the following reference: Lauer *et al.*, *Proc. Natl. Acad. Sci. USA*, 98, 12814-12819, 2001 (attached hereto as **Exhibit C**). Lauer *et al.* teach that no vasodilation occurs at venous plasma nitrite concentrations of 130 μM (see page 12816, column 2, last paragraph) and that physiological levels of nitrite are vasodilator-inactive (see the abstract). Because this reference was published in PNAS, scientific researchers in the nitric oxide field are likely read and trust the studies described in Lauer *et al.*.

8. The teachings of Lauer *et al.* are consistent with my understanding of inorganic nitrite physiology prior to October 14, 2003, which was that high concentrations of non-acidified sodium nitrite (for instance, concentrations above 200 μM) might cause vasodilation *in vitro*, but


lower concentrations (*e.g.*, below 25 μM) were completely vasodilator inactive both *in vitro* and *in vivo*. Prior to October 14, 2003, I believed that inorganic nitrite was an inert oxidation product of nitric oxide metabolism. I believe that my understanding of sodium nitrite physiology prior to October 14, 2003 accurately reflects the understanding of researchers working in the field at that time.

9. Thus, prior to October 14, 2003, I would not have expected sodium nitrite to have any beneficial therapeutic effect to induce vasodilation or increase blood flow when administered (for instance by injection or inhalation) to a subject at circulating concentrations of 25 μM or less, regardless of the *in vitro* results in rat aorta provided by Modin *et al.*.

10. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true; and further, these statements were made with the knowledge that willful false statements and like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date

09/14/09


Louis Ignarro, Ph.D.